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WAR DEPARTMENT

PHYSICAL SCIENCES DIVISION

CHEMICAL CORPS BIOLOGICAL LABORATORIES

Project No. 4-61-14-001

Report No. 21

15 April to 30 November, 1951

Project on Marine Biology DA-18-064-CML-471

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Conferences

9 June, 1951	Dr. E. J. Schantz
29 June, 1951	Dr. H. T. Cole, Executive Director, and Lt. Comdr. F. A. Chevrefils visited the project

University of California

The George Williams Hooper Foundation

San Francisco, Calif.

Mussels

Field Studies

In April the periodic sampling of the mussel beds was started. The samples were collected as far north as Fort Bragg and as far south as Big Sur. There are 21 sampling areas.

Summary:

21 locations

159 samples collected

35 round trips, or 9,594 miles

Seasonal high

Gualala	2880 Mu/50 gm mussel	July 5, 1951
Collection July 6	2,017,700 Mu in 18 buckets	

General trends

North: High in early July with a rapid drop. Short rise toward middle of August and constant low thereafter.

South: High in May in San Francisco area and second rise in mid July, but never over 500 Mu per 50 gm mussel. Points south of Green Canyon low throughout season.

On July 6 at Gualala 135 miles north of San Francisco the toxicity was found to be 2880 Mu/50 gm mussel. As the next day was the last low tide of the period, the other groups were not notified.

Collection

July 7 an 18-bucket collection was made at Gualala and returned to the laboratory for processing. The toxicity of these mussels was 2222 Mu/50gm mussel. The livers were removed and immediately transferred to 50% ethanol

acidified with 1 ml conc. HCl/liter. In this stage of extraction the poison was allowed to stand for 52 hours with 8 hour checks on the pH of the mixture. The pH was maintained at less than 5.0 and more than 2.0 by adjustment with conc. HCl. On July 9, 1951, the mixture was ground in an electric meat grinder; it yielded a final volume of 15 liters of acidified alcohol-ground liver slurry. The pH of this slurry was checked and readjusted as above.

A standard toxicity test on this slurry, processed by boiling and readjustment to initial volume, gave a toxicity of 192 Mu/ml of supernatant. The non-toxic, extracted residue represented 30% of the volume.

3000 ml of the slurry were removed and set aside for a stability test. This was adjusted once on July 9, 1951, to a pH of less than 5.0 and more than 2.0 by the addition of conc. HCl. Subsequent daily pH checks were made, but no additional acid was required. At the end of 27 days the toxicity of the supernatant, after boiling and readjustment to initial volume, was 192 Mu/ml.

The remaining 12 liters of material were made into a Celite 520-liver slurry mixture, packed into 8 liter percolating vessels, and extracted with 50% ethanol acidified with 1 ml of conc. HCl/liter. Percolate 1 consisted of 5 liters at 133 Mu/ml; percolate 2 was 5 liters at 133 Mu/ml; and percolate 3 was 5 liters at 57 Mu/ml. Successive percolates contained less than 20 Mu/ml of poison. The percolates were checked for a pH of less than 5.0 and more than 2.0 and adjusted with conc. HCl if necessary. Daily pH checks were done on this material.

The 3 liters of acidified alcohol-ground liver slurry and the 15 liters of percolate were shipped to Doctor Schantz at Camp Detrick on August 9, 1951.

Starting material

Original toxicity	2222 Mu/50 gm mussel	
18 buckets		
approximately 100	50 gm mussels/bucket	- 1800 50 gm mussels
Estimated total poison		3,999,600 Mu

Processed material

Ground liver - Acidified alcohol slurry	- 15,000 ml
30% solids	- 4,500 ml
Toxicity of extracted supernatant	- 192 Mu/ml
Total poison in slurry (- 30% solid)	- 2,016,000 Mu
3,000 ml slurry for stability test	- 403,200 Mu
12,000 ml slurry percolated	- 1,612,800 Mu

Material shipped

3,000 ml slurry after 27 days storage		- 403,200 Mu
Percolate 1	5,000 ml at 133 Mu/ml	- 665,000 Mu
Percolate 2	5,000 ml at 133 Mu/ml	- 665,000 Mu
Percolate 3	5,000 ml at 57 Mu/ml	- 284,500 Mu
Total Percolate		1,614,500 Mu
Total poison shipped		2,017,700 Mu
% poison lost while whole livers in acidified alcohol (52 hours)		49.6%
% poison recovered from ground liver-acidified alcohol slurry		100.1%

Based on the above results, a more efficient collection procedure is needed. The principal poison loss occurs while the livers are intact in spite of the acidified alcohol mixture. Immediate grinding of the livers after removal from the mussels is necessary. This can be accomplished on the beaches with hand grinders. The pH of the ground liver-acidified ethanol slurry must be checked at regular intervals and readjusted if necessary with conc. HCl to a pH of less than 5.0. The slurry sample handled in this manner suggested that after the initial pH readjustment, subsequent additions of acid will not be necessary. With regular pH checking, this stage of the poison extract is stable for at least a month and offers a convenient form for shipping with its smaller volume than that of the percolates.

Plankton Studies

Field work

With the increase in toxicity of the mussels, a few Gonyaulax were found in the water samples. There was never at any time visible "red water". At no time were there enough Gonyaulax present to make it feasible to think of concentrating them in view of extracting the poison directly.

Laboratory

The large refrigerated tank described in Report 20 was completed and the first large bottle was planted June 26, 1951, with 400 ml (of a large flask culture) to which was added 3400 ml of media No. 7. At the end of 7 days 16,800 ml of media were again added.

The first harvest was disappointing. With a total of 19,600 ml only 5,840 Mu were collected.

In view of past experiments it would appear that the method of aeration is not sufficient for the best growth.

Three different methods of aeration have been tested. Method I is still used as standard since it yields the best growth and is simplest. Method II, which is more unwieldy, yields as good growth. Method III yields poor growth.

I. Air from low pressure pump is bubbled through sterile distilled water and subsequently circulated over the surface of the culture.

II Sterile media is aerated by vigorous agitation at high pressure and allowed to flow slowly 50 ml/hour through dialysis tubing immersed in the culture.

III. Sterile media, vigorously aerated in a reservoir, is added to the culture at a constant rate to yield a final 1-7 dilution of original plant.

A fourth method, direct aeration, is now in progress and may give better results.

The experimentation with the cultures of Gonyaulax is being carried out in test tubes, 10-liter bottles, Fernbach culture flasks and 20-liter carboys.

A series of test tube cultures tested over a period of 6-10 days showed that Media No. 6 and a 7-day-old culture gave the best results. It takes 0.89 ml to equal a mouse unit or 3,180 Gonyaulax in an actively growing 7-day-old culture to produce one mouse unit. The Fernbach culture flask yields no better growth than the carboy-shaped bottles.

In the 20-liter carboys the best results were 2.2 ml/Mu or 5,070 Gonyaulax to produce a mouse unit. The total poison harvested from 120,800 ml in 7 bottles was 36,100 Mu.

K. A. Meyer